

**BIOSYSTEMATIC STUDIES ON THE *CLOSTERIUM*
MONILIFERUM/*EHRENBERGII* COMPLEX
(CHLOROPHYTA, CONJUGATOPHYCEAE)
IN WESTERN EUROPE. III. MORPHOLOGICAL VARIABILITY**

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ABSTRACT. — The morphological variability of 278 isolates of *Closterium ehrenbergii* and *C. moniliferum* originating from 17 populations in The Netherlands and England was analysed under uniform culture conditions. Dutch *C. ehrenbergii* isolates (mean cell length 376 μm , mean width 70 μm , mean curvature 112°) take an intermediate position between English *C. ehrenbergii* isolates (\bar{L} : 529 μm , \bar{W} : 92 μm , C : 104°) and all *C. moniliferum* isolates (\bar{L} : 226 μm , \bar{W} : 42 μm , C : 114°). This supports the notion based on previous biochemical analyses and mating experiments, that the Dutch *C. ehrenbergii* isolates belong to another biological entity than the English ones, more precisely, have a closer relationship to the *C. moniliferum* isolates.

RÉSUMÉ. — La variabilité morphologique de 278 souches des *Closterium ehrenbergii* et *C. moniliferum* isolées de 17 populations en Hollande et en Angleterre est analysée dans des conditions de culture uniformes. Les souches de *C. ehrenbergii* isolées en Hollande (longueur moyenne de la cellule 376 μm , largeur moyenne 70 μm , courbure moyenne 112°) occupent une position intermédiaire entre les souches de *C. ehrenbergii* isolées en Angleterre (\bar{L}_0 : 529 μm , \bar{L}_a : 92 μm , C : 104°) et l'ensemble des souches de *C. moniliferum* (\bar{L}_0 : 226 μm , \bar{L}_a : 42 μm , C : 114°). Ceci renforce la notion, déjà acquise grâce aux analyses biochimiques et aux expériences sur la reproduction sexuée, que les souches de *C. ehrenbergii* isolées en Hollande appartiennent à une autre entité biologique que les souches de *C. ehrenbergii* isolées en Angleterre, et plus précisément qu'elles sont plus proches des souches de *C. moniliferum*.

KEY WORDS : biosystematics, morphological variability, desmids, *Closterium moniliferum*, *Closterium ehrenbergii*.

INTRODUCTION

In a previous paper (Coesel & Menken, 1988) we drew the attention to marked differences in isozyme patterns between a number of Dutch and English populations of *Closterium ehrenbergii* Menegh. ex Ralfs. In fact, much more

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resemblance could be established between Dutch populations of *Closterium ehrenbergii* and Dutch or English populations of *Closterium moniliferum* (Bory) Ehr. ex Ralfs than between Dutch populations of *C. ehrenbergii* and English ones of that same typological species. The low phenotypic identity in isozymes between the Dutch and English *C. ehrenbergii* populations indicate intrinsic genetic differences. Indeed, it was not possible to cross Dutch strains of *C. ehrenbergii* sexually with English ones. While all 66 *C. ehrenbergii* clones from English populations appeared, as plus or minus strains, to male part of one single mating group, we did not succeed in inducing any sexual activity at all in 90 Dutch clones of *C. ehrenbergii* (Coesel, 1988).

While all these 156 clones on the basis of cell shape, cell dimensions and arrangement of pyrenoids unmistakably refer to the typological species *C. ehrenbergii*, some more or less consistent morphological differences between the Dutch and the English populations can be observed. These morphological differences are the topic of this paper.

MATERIAL AND METHODS

The material investigated consists of 156 clones of *C. ehrenbergii* originating from four English (A-D) and five Dutch (E-I) populations, and 122 clones of *C. moniliferum* from two English (J-K) and six Dutch (L-Q) populations. For details concerning the sampling stations, see Coesel & Menken (1988), for culture conditions of the clones Francke & Coesel (1985).

The clones were morphologically screened in a stationary growth phase. From each clone 25 cells were randomly selected for measuring of length (distance between apices to $\pm 5 \mu\text{m}$), width (maximum value in μm at the mid region) and curvature. The degree of curvature was assessed to the nearest 5° interval using the so-called Closterio-curvimeter devised by Heimans (1946), by superposing it over the microscopic image of the cell obtained with a drawing-tube. Moreover, from a survey of hundreds of cells in each clone, the upper and lower limits for length, width and curvature were established (obvious rare anomalous growth forms were excluded).

RESULTS

The mean values for cell length, cell width and curvature obtained from a random test of 25 cells out of each clone were used for calculating the data per population as presented in Table 1. For each of the populations of *C. ehrenbergii* and *C. moniliferum* the mean values with standard deviation have been assessed by averaging over the mean values of the individual clones. In addition extreme values for the three parameters, detected by inspecting large lots of cells are supplied. These extreme values may be useful in evaluating the discriminating significance of a given parameter.

Spec. Pop.	No. of clones	Cell length (μm)			Cell width (μm)			Curvature ($^{\circ}$)			
		Mean	SD	Extremes	Mean	SD	Extremes	Mean	SD	Extremes	
<i>C. ehr.</i>	A	3	555	51.5	455-675	102	5.9	95-114	107	11.1	85-125
	B	16	501	40.5	380-630	85	5.2	76-100	105	9.8	85-140
	C	35	520	46.7	410-700	88	5.9	74-105	98	7.5	80-120
	D	12	541	68.8	365-725	91	4.9	84-103	105	6.3	85-125
	E	8	375	20.1	310-455	67	1.5	63- 72	111	3.5	90-120
	F	27	385	20.0	300-485	72	5.6	59- 82	113	5.6	90-135
	G	5	366	21.0	320-410	74	2.9	69- 80	112	5.4	95-130
	H	9	374	11.9	315-420	70	1.7	65- 74	116	7.2	95-135
	I	41	380	20.3	310-460	65	4.6	57- 76	110	8.5	90-150
<i>C. mon.</i>	J	21	214	17.5	140-265	36	3.2	30- 40	109	4.4	90-130
	K	4	242	26.7	190-285	42	7.2	32- 49	109	13.0	90-135
	L	6	226	17.1	190-280	39	4.8	34- 46	106	10.6	85-125
	M	12	207	16.0	180-260	42	5.4	31- 49	111	10.1	85-140
	N	34	233	17.0	175-275	44	3.7	34- 48	126	8.6	95-155
	O	17	237	13.1	195-275	46	1.9	40- 49	119	10.6	95-160
	P	24	227	18.0	180-280	45	2.2	40- 51	118	11.9	90-150
	Q	4	219	10.9	185-260	43	2.5	40- 48	115	12.7	90-135

Table I — Mean values with standard deviation per population of *C. ehrenbergii* and *C. moniliferum* for cell length, cell width and degree of curvature. Also the most extreme values measured in any of the clones belonging to a given population are stated.

From Table I at first glance a marked difference in dimensions is clear between *C. ehrenbergii* and *C. moniliferum*, which were primarily identified on the basis of arrangement of pyrenoids. Within the *C. moniliferum* clones cell length is always less than 300 μm . This seems to be the lower limit for cell length in *C. ehrenbergii*. Likewise, in the material under discussion a cell width of ca. 55 μm can be assigned as an absolute dividing line between the two species. The clear discrimination of these two dimensions contrasts with a large overlap of the degree of curvature found in the two species. Besides discriminating between the two species, these measures also reveal differences between Dutch and English populations of *C. ehrenbergii*. This is seen when the values from Table I are graphically represented in scatter diagrams. Fig. 1 in which mean cell length for all individual populations is plotted against cell width, three distinct clusters are discernable. One of these, showing no overlap at all, concerns all clones of *C. moniliferum*. The other ones refer to all Dutch *C. ehrenbergii* clones and

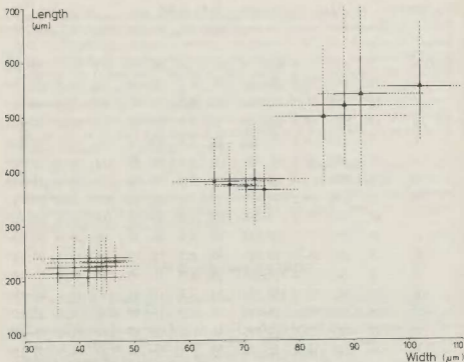


Fig. 1 — Diagram showing variability in cell length and width in populations of *C. moniliferum* (mean values indicated as dots), *C. ehrenbergii* from Holland (mean values indicated as squares), and *C. ehrenbergii* from England (mean values indicated as triangles). Solid lines indicate standard deviations, dotted lines extreme values encountered in any of the clones belonging to a given population.

all English *C. ehrenbergii* clones respectively. The phenotype clusters corresponding to Dutch and English *C. ehrenbergii* respectively are completely separated when mean population values with standard deviations are considered. If the extreme values found in hundreds of cells of each clone are included, there is some overlap, especially in cell length. A similar, if less obvious, distinction between Dutch and English populations of *C. ehrenbergii* can be noted with respect to the degree of curvature. Fig. 2 in which curvature is plotted against cell length, shows that the mean curvature values for the English populations are all lower than these for the Dutch ones. However, there is considerable overlap in the individual values per population as seen by the standard deviations.

These differences in morphology between Dutch and English *C. ehrenbergii* populations are also manifest from Figs. 3-8 showing photographs of cells from

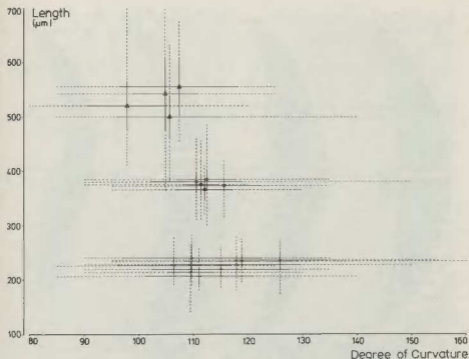


Fig. 2 — Diagram showing variability in cell length and curvature in populations of *C. moniliferum* (dots), *C. ehrenbergii* from Holland (squares) and *C. ehrenbergii* from England (triangles). Symbols as in Fig. 1.

both groups as compared with some *C. moniliferum* clones. Apart from differences in absolute cell length and width, corresponding to the three groups in question, there are also differences in cell slenderness (length to width ratio). However, these are not fully congruent with the above mentioned groups. Length to width ratios per population range from 5 to 6 within each of the three morphological groups: Dutch *C. ehrenbergii*, English *C. ehrenbergii* and *C. moniliferum* (Table II). Within *C. ehrenbergii*, there is a slight tendency in the English populations for a somewhat higher ratio than in the Dutch ones.

DISCUSSION

The present results make clear that on the basis of the available clones *C. ehrenbergii* from England can be morphologically distinguished from Dutch clones of that species. In fact the Dutch *C. ehrenbergii* clones that in their isozyme

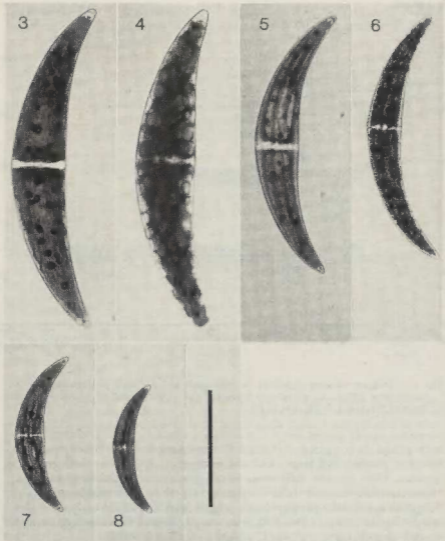


Fig. 3-8 - Photographs showing morphological variability. Figs. 3-4 : *C. ehrenbergii* from England (clones nos 837 and 902, from populations D and C respectively). Figs. 5-6 : *C. ehrenbergii* from Holland (clones nos 241 and 1025, from populations E and I respectively). Figs. 7-8 : *C. moniliferum* (clones nos 1052 and 782, from populations P and J respectively). Scale bar : 200 μ m.

banding patterns much more resemble *C. moniliferum* than the English *C. ehrenbergii* clones do (Coesel & Menken, 1988), appear also in a morphological

Pop.	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
L/W	5.4	5.9	5.9	5.9	5.5	5.4	5.0	5.3	5.9	6.0	5.9	5.9	5.0	5.3	5.1	5.1	5.1
SD	0.2	0.4	0.4	0.6	0.3	0.5	0.2	0.2	0.6	0.5	0.5	0.8	0.5	0.4	0.2	0.2	0.2

Table II — Mean cell length to width ratio with standard deviation per population of English *C. ehrenbergii* (A-D), Dutch *C. ehrenbergii* (E-I), English *C. moniliferum* (J-K), and Dutch *C. moniliferum* (L-Q).

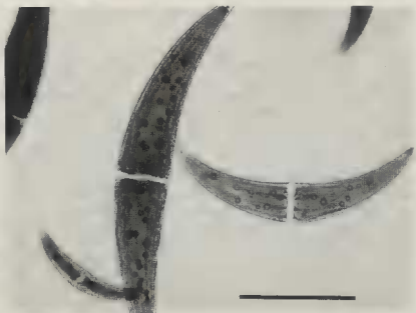


Fig. 9 — Photograph illustrating the intermediate morphological character of *C. ehrenbergii* from Holland (clone no 245 from population E, right in the picture) as compared to *C. ehrenbergii* from England (clone no 837 from population D, mid in picture) and *C. moniliferum* (clone no 744 from population J, lower left). Scale bar: 200 μ m.

sense nearer to *C. moniliferum* than the English ones do. The intermediate character in dimensions of Dutch *C. ehrenbergii* as compared with *C. moniliferum* and English *C. ehrenbergii* is especially well illustrated in Fig. 9 where the three types are shown next to each other.

The morphological differences between English and Dutch populations of *C. ehrenbergii* in addition to the previously established differences in isozymes

	Cell length (μm)			Width (μm)			Curvature ($^{\circ}$)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
<i>C. ehrenbergii</i> -England	501-555	529	23.7	85-102	92	7.4	98-107	104	3.9
<i>C. ehrenbergii</i> -Holland	366-385	376	7.1	65-74	70	3.6	110-116	112	2.3
<i>C. moniliferum</i>	207-242	226	11.8	36-46	42	3.3	106-126	114	6.6

Table III — Morphological characteristics, based on mean population values, for the three clusters distinguished within the *Closterium* clones investigated.

and sexual compatibility support the notion that this typological species in western Europe comprises several biological entities. This has also been found for Asiatic (mainly Japanese) populations of *C. ehrenbergii* by Ichimura and co-workers (e. g. Ichimura, 1985). When comparing our morphological data with those supplied by Kasai & Ichimura (1986) for three different mating groups of *C. ehrenbergii*, comprising strains from Japan, Nepal, Taiwan and Australia, the relatively small cell dimensions in those mating groups are notable. The mean length (404 μm) and width (67 μm) values in their mating group B are in the range of our Dutch *C. ehrenbergii* clones while the mean cell sizes in their mating groups H (\bar{l}_g : 333 μm , \bar{w} : 57 μm) and especially A (\bar{l}_g : 250 μm , \bar{w} : 50 μm) approach those of our *C. moniliferum* clones (Table III). On the other hand the large cell dimensions of our English *C. ehrenbergii* clones are rarely found in the Asiatic material, i. e. two Taiwanese strains belonging to their mating group B. However, compared with those two strains our English *C. ehrenbergii* clones are distinctly less curved and less slender (see figs. 3m-n in Kasai & Ichimura, l. c.). A valid assessment of the relationship between the *C. ehrenbergii* clones dealt with in this paper and the Asiatic ones studied by Ichimura c. s. will have to await a more detailed investigation of the geographical distribution and ecology of the so-called Dutch and English *C. ehrenbergii* types in western Europe.

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